Cellular Immunity to Rous Sarcoma in Tumor-bearing Chickens

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SUMMARY

Chickens carrying primary sarcomas induced by Schmidt-Ruppin Rous virus were studied for possible cellular immunity against the tumor-specific transplantation antigens of those tumors by use of the colony inhibition test. The thymus cells of 7 out of 8 tested chickens were found to reduce significantly the colony formation of Rous sarcoma cells while they had no similar effect on other types of tumors tested in parallel. It was further shown that thymus cells which had been frozen, stored in liquid nitrogen for 3 months, and subsequently thawed kept their specific colony-inhibiting activity. The implications of these findings are discussed.

INTRODUCTION

Although it is generally agreed that experimental neoplasms induced by viruses or chemical carcinogens possess TSTA, the opinion is still conflicting whether or not animals carrying primary tumors contain lymphoid cells sensitized against the tumor antigens. On one hand, data by Mikulska et al. (17) utilizing the Winn neutralization test indicate that spleen cells from mice carrying chemically induced tumors are nonreactive, and CI tests of Barski and Youn (4), performed with peritoneal lymphocytes support the findings of Mikulska et al. This lack of cellular immunity in the primary tumor animals versus the TSTA in question has been suggested to be of primary importance for the progressive growth and spread of tumor (1). On the other hand, Allison performed neutralization tests, similar to those of Mikulska et al., using adenovirus-induced tumor cells as targets and found that lymphocytes from tumor-bearing animals were reactive (2). There have also been a number of recent reports from CI tests which indicate that animals carrying progressively growing tumors do contain lymph node cells which are cytotoxic to the autochthonous tumor cells or to other tumor cells carrying the same TSTA. The first of these reports showed that mice carrying primary methylcholanthrene-induced sarcomas had a cellular immunity to TSTA and occasionally also had cytotoxic antitumor antibodies (9). Lymph node cells specifically reacting against the TSTA of primary tumors were also demonstrated in mice carrying primary Moloney sarcomas (7) or mammary carcinomas (12), in rabbits carrying primary Shope papillomas (8), and in rats carrying primary methylcholanthrene-induced sarcomas, polyoma tumors, or RSV-SR-induced sarcomas (10, 15, 18, 19).

This paper reports the results of similar studies on RSV-SR-inoculated chickens carrying primary sarcomas. Thymus cells of the tumor-bearing animals were tested in vitro for CI activity against RSV-SR sarcoma cells, since the avian thymus is known to be primarily engaged in the development of lymphocytes which are mediators of cell-bound immunity reactions (5, 22).

MATERIALS AND METHODS

Chickens. The chickens were derived from an isolated leukemia-free stock of White Leghorn (Vinterled, Laagesta, Sweden). Embryos from this stock react negatively in the COFAL test (13), for the leukemia group of viruses and embryos are furthermore highly and almost uniformly susceptible to RSV-SR in vitro (14).

Virus Inoculation. Chickens were inoculated into the pectoral muscles and wing web with 0.25 ml of a RSV-SR pool (titer, 15 X 10⁵ FFU/ml) heated to 56° for 1 hr. The virus pool was prepared as previously described (16) and essentially consisted of the supernatant of a centrifuged (350 x g) homogenate of chicken RSV-SR sarcoma tissue. The purpose of the heat treatment was to inactivate the virus partially in order to obtain slow-growing tumors after a long latency period. Three chickens (Chickens 67, 69, and 70) received no further treatment, while 5 chickens (Chickens 57, 58, 61, 62 and 63) received 2 to 4 more 0.1-ml doses of the RSV-SR pool in the pectoral muscles. All the chickens except one (Chicken 58) developed progressively growing Rous sarcomas in the pectoral muscles as well as the wing webs. Chicken 58 developed only temporarily small palpable nodules which later regressed. Control chickens were immunized in parallel with homogenates of normal muscle tissue prepared in the same way as the virus pool.

Thymus Cells. After decapitation of the chickens, thymus lobes were removed, and cell suspensions for the CI test were prepared by pressing the thymus tissue through a 60 mesh stainless steel screen into MEM and washing the suspensions with MEM.

CI Technique. The procedure used to detect thymus cell-mediated immunity followed the method described previously for demonstration of lymph node cell-mediated immunity (6, 11). Trypsinized suspensions of tumor cells were added to
Cellular Immunity in Tumorous Chickens

60-mm plastic Petri dishes (Falcon Plastics, Los Angeles, Calif.) at 150 to 250 cells/dish. From 12 to 24 hr later, the medium was removed, and 0.5 ml of a suspension containing 10^7 thymus cells in MEM was added to each dish (4 to 5 dishes/group). The dishes were incubated for 2 hr at 37° in a 5% CO_2 in air atmosphere, after which 3 ml of medium containing 15% fetal calf serum were added, and the dishes were then incubated for 3 to 5 days at 37°. The percentage reduction of colony numbers was calculated by comparing the colony numbers obtained with thymus cells from chickens treated with allogeneic normal tissue homogenates or with such cells from untreated animals.

Tumors. The following tumors were used as target cells in the CI tests: Sarcoma RSC induced with RSV-SR in the mouse strain A/Sn and kept in serial passages in vivo (10 passages) and in vitro for 3 months; Control tumor NIA originating from A/Sn mouse embryo cells by spontaneous neoplastic transformation in vitro (J. Ponten, unpublished observations). It grew progressively after inoculation into syngeneic mice and formed fibroblastic, rapidly growing tumors. The Moloney polyoma mouse sarcoma clone YAA-RC2 of A/Sn origin (20) and the A12B8 adenovirus type 12 tumor induced in a CBA mouse (3) were 2 further control tumors.

Freezing Procedure. Suspensions of thymus cells in MEM containing 50% fetal calf serum and 10% dimethyl sulfoxide were distributed into vials frozen down slowly (approximately 1 to 1.5°/min) to about −70°, and transferred into a liquid nitrogen storage container. The vials were kept in liquid nitrogen for 3 months. The cell suspensions were rapidly thawed in a 37° water bath and diluted in MEM; the cells were washed once, counted after trypan blue staining, and used in a CI test (Table 1, Experiment 2). The percentage of stained cells was 25 to 45% compared to 2 to 12% in the fresh suspensions.

Table 1
Effect of thymus cells from RSV-SR inoculated and control chickens on colony formation by Rous and non-Rous target tumor cells

<table>
<thead>
<tr>
<th>Thymus cell&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Target tumor cells</th>
<th>Chicken Donor No.</th>
<th>Pretreatment</th>
<th>No. of colonies/dish</th>
<th>% reduction&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSC (250 cells/Petri dish)</td>
<td>1</td>
<td>None</td>
<td>106 ± 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>57</td>
<td></td>
<td>62 ± 2.4</td>
<td>42**</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>61</td>
<td></td>
<td>86 ± 3.9</td>
<td>19*</td>
<td></td>
</tr>
<tr>
<td>RSC (225 cells/Petri dish)</td>
<td>2</td>
<td>None</td>
<td>25 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>3</td>
<td>Normal chicken tissue</td>
<td>24 ± 2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>57</td>
<td></td>
<td>13 ± 0.5</td>
<td>48**</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>61</td>
<td></td>
<td>15 ± 1.6</td>
<td>40**</td>
<td></td>
</tr>
<tr>
<td>NIA control tumor (200 cells/Petri dish)</td>
<td>2</td>
<td>None</td>
<td>34 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>3</td>
<td>Normal chicken tissue</td>
<td>36 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>57</td>
<td></td>
<td>36 ± 3.4</td>
<td>−6</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>61</td>
<td></td>
<td>39 ± 2.6</td>
<td>−15</td>
<td></td>
</tr>
<tr>
<td>RSC (225 cells/petri dish)</td>
<td>4</td>
<td>Normal chicken tissue</td>
<td>88 ± 4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>58</td>
<td></td>
<td>87 ± 3.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>63</td>
<td></td>
<td>69 ± 3.8</td>
<td>22**</td>
<td></td>
</tr>
<tr>
<td>A12B8 adeno 12 tumor (150 cells/Petri dish)</td>
<td>4</td>
<td>Normal chicken tissue</td>
<td>101 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>58</td>
<td></td>
<td>101 ± 7.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>63</td>
<td></td>
<td>99 ± 1.7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>RSC (250 cells/Petri dish)</td>
<td>5</td>
<td>None</td>
<td>142 ± 5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>6</td>
<td>Normal chicken tissue</td>
<td>143 ± 5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>62</td>
<td></td>
<td>113 ± 5.1</td>
<td>20**</td>
<td></td>
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<tr>
<td>YAA-RC2 control tumor (200 cells/Petri dish)</td>
<td>6</td>
<td>Normal chicken tissue</td>
<td>10 ± 2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (repeated doses)</td>
<td>62</td>
<td></td>
<td>13 ± 2.4</td>
<td>−30</td>
<td></td>
</tr>
<tr>
<td>RSC (250 cells/Petri dish)</td>
<td>7</td>
<td>None</td>
<td>50 ± 3.5</td>
<td></td>
<td></td>
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<tr>
<td>RSV-SR (single dose)</td>
<td>67</td>
<td></td>
<td>31 ± 1.9</td>
<td>38**</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (single dose)</td>
<td>69</td>
<td></td>
<td>33 ± 3.3</td>
<td>34**</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (single dose)</td>
<td>70</td>
<td></td>
<td>32 ± 2.6</td>
<td>36**</td>
<td></td>
</tr>
<tr>
<td>A12B8 adeno 12 tumor (150 cells/Petri dish)</td>
<td>7</td>
<td>None</td>
<td>41 ± 3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV-SR (single dose)</td>
<td>67</td>
<td></td>
<td>45 ± 1.5</td>
<td>−12</td>
<td></td>
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<tr>
<td>RSV-SR (single dose)</td>
<td>69</td>
<td></td>
<td>44 ± 3.7</td>
<td>−7</td>
<td></td>
</tr>
<tr>
<td>RSV-SR (single dose)</td>
<td>70</td>
<td></td>
<td>43 ± 1.9</td>
<td>−5</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The thymus cells were added at 10^7 cells/dish.

<sup>b</sup> The probability that the difference between colony numbers in experimental and control group is due to chance is indicated. *, p < 5%; **, p < 1%.

<sup>c</sup> In Experiment 2, thymus cells were used after storage in liquid nitrogen for 3 months.
The demonstration that thymus cells of chickens bearing a primary RSV-SR sarcoma do react specifically with RSV-SR tumor cells shows that there is a cellular immunity also in chickens against the TSTA of primary tumors. This finding extends the previously obtained evidence for a comparatively strong cellular immunity against TSTA in various animals carrying primary, progressively growing tumors. The progressive growth of primary antigenic tumors thus appears to be due not to a lack of cellular immunity but rather to some mechanisms (such as appearance of blocking antibodies) by which the tumor cells are protected from this immunity or possibly to a faster growth than elimination rate. The former possibility is suggested by the finding that animals bearing various types of primary tumors have such blocking antibodies (7-10, 12, 18, 19), while the blocking activity cannot be detected before tumor development (10, 18, 19) at a time when cellular immunity is demonstrable (18, 19).

It was shown that thymus cells which had been frozen down, stored in liquid nitrogen for 3 months, and subsequently thawed kept their ability to inhibit specifically the colony formation of Rous sarcoma cells. It has also been shown that similarly frozen and thawed thoracic duct lymphocytes of rats bearing primary RSV-SR sarcomas maintain their immunity to the TSTA of RSV-SR tumor cells as measured by the CI test (K. Borum and H. O. Sjögren, unpublished observations). These results show that sensitized lymphocytes may be stored for prolonged periods of time with maintained immune activity, which will greatly facilitate the assay of their antitumor activity.

Cellular immune competence of chickens is mediated by thymus and spleen cells, while the bursa is primarily concerned with humoral antibody synthesis (5). As a consequence, cellular immunity and humoral antibody formation may be separately suppressed in chickens by bursectomy or thymectomy and X-irradiation. It therefore appears feasible to use the chicken Rous sarcoma model to throw some further light on the mechanisms (such as appearance of blocking antibodies) by which the tumor cells are protected from this immunity or possibly to a faster growth than elimination rate. The former possibility is suggested by the finding that animals bearing various types of primary tumors have such blocking antibodies (7-10, 12, 18, 19), while the blocking activity cannot be detected before tumor development (10, 18, 19) at a time when cellular immunity is demonstrable (18, 19).

ACKNOWLEDGMENTS

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REFERENCES


RESULTS

Table 1 shows the effects of chicken thymus cells on the colony formation by Rous mouse target tumor cells (RSC) and by different non-Rous target tumor cells.

The thymus cells from 7 out of the 8 RSV-SR-inoculated chickens (Chickens 57, 61, 62, 63, 67, 69, and 70) reduced the colony formation by Rous mouse target tumor cells (RSC) and was obtained with thymus cells from any of the RSV-SR-inoculating effect is further illustrated by the fact that no effect was demonstrated with thymus cells from chickens had tumors with a diameter of 2 to 4 cm in the pectoral muscles and wing webs.

No CI was demonstrated with thymus cells from chickens treated with normal chicken cells. The specificity of the inhibiting effect is further illustrated by the fact that no effect was obtained with thymus cells from any of the RSV-SR-inoculated chickens when different non-Rous tumor cells were used as target cells.

DISCUSSION

It has been demonstrated previously that RSV-SR sarcomas derived from mice, rats, hamsters, and chickens have common TSTA (19, 21). This was the basis for the use of a mouse RSV-SR target tumor in the present study of cell-bound immunity against RSV-SR TSTA in chickens carrying primary Rous sarcomas. By using this mouse tumor rather than any chicken tumor, it was possible to perform the test on a tumor with high plating efficiency and known demonstrable RSV-SR-specific TSTA. Furthermore, adequate control tumors induced by other means were available for parallel tests. There was no evidence of any nonspecific reactions in the controls performed, i.e., immunization of chickens against normal chicken tissue did not lead to any reactivity of the thymus cells against mouse target cells nor did thymus cells of tumor-bearing chickens react against any of the control mouse tumor cells.

The demonstration that thymus cells of chickens bearing a primary RSV-SR sarcoma do react specifically with RSV-SR tumor cells shows that there is a cellular immunity also in chickens against the TSTA of primary tumors. This finding extends the previously obtained evidence for a comparatively strong cellular immunity against TSTA in various animals carrying primary, progressively growing tumors. The progressive growth of primary antigenic tumors thus appears to be due not to a lack of cellular immunity but rather to some mechanisms (such as appearance of blocking antibodies) by which the tumor cells are protected from this immunity or possibly to a faster growth than elimination rate. The former possibility is suggested by the finding that animals bearing various types of primary tumors have such blocking antibodies (7-10, 12, 18, 19), while the blocking activity cannot be detected before tumor development (10, 18, 19) at a time when cellular immunity is demonstrable (18, 19).

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